



COMPARATIVE ANALYSIS OF THE ANTIMICROBIAL EFFICACY OF AYURVEDIC SEEDS IN WASTEWATER FILTRATION

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ABSTRACT

Waterborne diseases such as *Hepatovirus*, *Vibrio Cholera*, *Shigella*, *Salmonella*, *Campylobacter*, and *Escherichia coli* pose significant public and individual health challenges in underdeveloped areas lacking access to safe and clean drinking water, as mentioned in the UN Sustainable Development Goal Number 6, which highlights the need for sustainable and cheap water purification methods. This experiment tested the antimicrobial properties of the following ayurvedic (i) *Strychnos Potatorium*, (ii) *Moringa Oleifera*, (iii) *Salvia hispanica*, (IV) *Foeniculum vulgare* Mill, by taking stable concentrations of each seed extract and testing them against dirty water samples collected from polluted water sources. Water samples have been mixed with definite concentrations of extract, spread under agar, and then incubated for 24 hours to identify bacterial inhibition zones. After 24 hours of incubation, a Manual colony counter LMCC-A11 was used to identify major countable colonies. The sample of *Strychnos Potatorium* showed the greatest bacterial inhibition, followed by *Foeniculum Vulgare* Mill, *Slavia Hispanica*, and *Moringa Oleifera*. Compared to dirty water, the seeds inhibited what was nearly 500-700 colonies of bacteria that could have been present in the samples. With the results above, seeds, ayurveda sample *Strychnos Potatorium*, showed the most antimicrobial activity against the various bacteria and harmful compositions present in dirty water, representing its strong potential in being able to filter microbes in water to greater extents. This investigation addresses the importance of sustainable and cheaper methods of water filtration by diving into the antimicrobial aspects of various plant extracts against contaminated water, highlighting its replaceability with traditional filtration methods

KEYWORDS: Immediately After the Abstract, Provide a Maximum of 6 Key Words

INTRODUCTION

Water purification, the unique process that encompasses the removal of undesired chemical compounds, forms a crucial backbone for most of our human activities today. With Earth's natural source of fresh water (Freshwater represents the use of water for farming, drinking, and washing) covering just 1.2% of Earth's land, early pioneers recognized the importance of developing effective water purification methods and ensuring that millions of people receive the advantages of the scarce fresh water.

However, water pollution is now one of the leading causes of diseases and deaths today. Even after water has been treated, inorganic, and organic pollutants and microscopic bacteria still enter water distribution systems and reach water sources.

The main sources of water contamination are farming, fossil fuel power plants, and wastewater. (*What Are the Main Sources of Water Pollution, N.D.*) There were 52.2 million tonnes to 215.3 million tonnes increase in fertilizer consumption between 1961 and 2019. Globally 2 tonnes of agricultural and industrial waste are disposed into water sources and about 84% of energy needs are met by the consumption of fossil fuels. (*The Lancet Commission on Pollution and Health, 2017*)

Over 80 percent of global wastewater runs back into the environment. Many strategies, including wastewater treatment

facilities and water restrictions, are used to abate the effects. However, despite all efforts, public health is still largely affected by inadequate access to clean water on a worldwide scale. Contaminated drinking water can transmit diseases and is estimated to cause approximately 505,000 deaths each year. (World Health Organization: WHO, 2023) The prevalent issue of water pollution is impacting human health.

To tackle this issue, this research paper focuses on investigating plant extracts of various ayurvedic seeds as antimicrobial alternatives, to expensive and unsustainable water filtering mechanisms. Extracts of *Strychnos Potatorium*, *Moringa oleifera*, *Salvia hispanica*, and *Foeniculum vulgare* Mill have been investigated against contaminated water from swamps of the *Shankarpalli Mandal District*. in search of an ideal seed-based water filter.

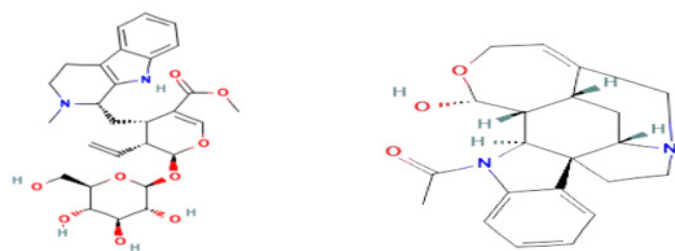
1.1 *Strychnos Potatorium*

In the vast field of Ayurveda, composing plants and seeds have served predominantly towards the rich field of Molecular Biology. The *Strychnos Potatorium* seed is an ancient Sanskrit seed that has been reported to clear turbid platforms of contaminated water. It originates from the Loganiaceae (*Strychnaceae*) family and has been employed widely by the native practitioners of Hindustan. The tree is home to forested areas of India, Sri Lanka, Burma, and Southern East Asia, and is more commonly referred to as the "clearing nut tree" or the

“nirmali” seed. (GJRA, “*Strychnos Potatorium* Seed Powder as a Water Purifier”, 2015).

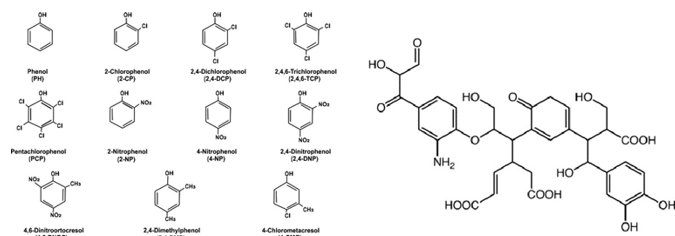
Extracts of the *Strychnos Potatorium* seed have been a native source of polysaccharides and several polymers aiding the process of coagulation in water filtration, coagulation being a crucial aspect in the side with antimicrobial properties. Anion polyelectrolyte is a water-soluble polymer that carries a negative charge, carrying the ability to effectively coagulate through the efficient binding of charged colloids, a unique process of interparticle Bridging. (IIIT Delhi, Coagulation and flocculation, 2011).

Alkaloid Fractions present in the seed such as Diaboline and Dolichantoside have various functional groups that increase floc size, treating the coagulation process more efficiently. (EJ-Chem, Phytochemical Studies of *Strychnos potatorium* L.f, 2007) Diaboline is a curare alkaloid extracted from the flowering plant *Strychnos diabolii* by H. King in 1949. (ACS, Diaboline, 2012). Dolichantoside is a glucoindole alkaloid present widely in the Loganiaceae family and the *Strychnos* Plant. (NIH, Main Alkaloid from Stem Bark of *Strychnos tricalysoides*, 1984). Below are the chemical structures of both alkaloids.



Dolichantoside Left (Pub Chem, *Dolichantoside*, 2006), *Diaboline* Right (Pub Chem, *Diaboline*, 2007)

Functional groups in the surface of Diachantoside and Diobline present in Sytrchnos Potatorium, preferably the Nitrogen Groups, Hydrogen Groups, and Oxygen Groups can attract particles present in dirty water samples such as phenolic compounds and humic substances. (Science Direct, Organic Contaminant, 2020) Below are the chemical structures of both compounds.



Phenolic Compounds Left (RG, Presence of phenol in wastewater effluent and its removal: an overview, 2020)

Humic Acid Right (RG, Characterization of humic acids from composted agricultural Saudi waste, 2018)

Humic Acid and Phenolic Compounds are produced from decomposed material in the soil, and they make contact with

water through runoff materials. Phenolic Compounds can contaminate water through sewage surfaces. Alkaloids are present in the *Strychnos P*. It can attract Humic Acid and Phenolic Compounds through its oxygen and hydrogen groups in the alkaloid functional groups. The phenomenon of adsorption or interaction of charged particles (dipole-dipole, hydrogen bonding, or ion-dipole interaction). These processes can lead to an increase in floc production, ascendingly increasing the rate of coagulation and clarifying the water more.

Strychnos P. also possesses a great antimicrobial intent, allowing this property to be of help in the water filtration process. Alcoholic Extracts of *Strychnos P*. at tested concentrations of (100 and 200 µg/ml) have inhibited the following, *Proteus vulgaris*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Vibrio cholerae*, *Mycobacterium tuberculosis*, *Aspergillus niger*, and *Candida albicans* significantly. The seed also exhibited antibacterial properties against *Staphylococcus aureus*, and *Escherichia coli* (NIH, *Strychnos potatorium*: Phytochemical and pharmacological review, 2014),

In another experiment and research paper conducted by P. B. MALLIKHARJUNA* and Y. N. SEETHARAM (EJ, In Vitro Antimicrobial Screening of Alkaloid Fractions from *Strychnos potatorium*, 2009), alkaloid fractions were extracted and tested against various bacteria in a Zone of inhibition like experiment. Below are the results.

Table 1. Antibacterial activity of *Strychnos potatorium* alkaloid fractions.

Drug, 100 µg/mL	Zone of inhibition diameter (mm) of bacteria						
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. vulgaris</i>	<i>P. aeruginosa</i>	<i>S. typhimurium</i>	<i>Klebsiella sp.</i>	<i>V. cholerae</i>
Streptomycin	18.33 (± 0.33) ^{a,c}	13.67 (± 0.33) ^a	15.33 (± 0.33) ^c	14 (± 0.58) ^b	14.67 (± 0.33) ^a	15.33 (± 0.67) ^b	15 (± 0.58) ^a
Brucine	12.67 (± 0.33) ^a	17 (± 0.58) ^c	17 (± 0.58) ^d	12.33 (± 0.33) ^a	16 (± 0.58) ^b	15.67 (± 0.33) ^b	17 (± 0.58) ^b
Total alkaloids	14.67 (± 0.33) ^b	17 (± 0.58) ^c	15.33 (± 0.33) ^c	14 (± 0.58) ^b	15.67 (± 0.88) ^b	16.67 (± 0.33) ^c	17.67 (± 0.33) ^b
PB - I	14 (± 0.58) ^b	17 (± 0.58) ^c	14 (± 0.58) ^b	13.67 (± 0.33) ^a	16 (± 0.58) ^b	12.33 (± 0.33) ^a	18.33 (± 0.33) ^b
PB - II	12 (± 0.33) ^a	16 (± 0.58) ^b	11 (± 0.58) ^a	14.33 (± 0.33) ^b	14.33 (± 0.33) ^a	13 (± 0.0) ^a	18.33 (± 0.33) ^b
PB - III	11.67 (± 0.33) ^a	15.33 (± 0.33) ^b	14 (± 0.58) ^b	14 (± 0.58) ^b	16.33 (± 0.33) ^b	14 (± 0.58) ^b	19.67 (± 0.33) ^c
PB - IV	12.33 (± 0.33) ^a	16.67 (± 0.33) ^b	17.67 (± 0.33) ^d	14.67 (± 0.33) ^b	16.67 (± 0.33) ^b	15.67 (± 0.33) ^b	19.33 (± 0.33) ^c
Diaboline	12.67 (± 0.33) ^a	17.33 (± 0.33) ^c	19 (± 0.58) ^a	14.33 (± 0.33) ^b	17 (± 0.58) ^c	14.33 (± 0.33) ^b	20 (± 0.58) ^c
PB - V	12 (± 0.33) ^a	17.33 (± 0.33) ^c	18.67 (± 0.58) ^a	13 (± 0.33) ^b	17 (± 0.58) ^c	12.33 (± 0.33) ^b	17 (± 0.58) ^c
PB - VI	11.67 (± 0.0) ^a	19 (± 0.67) ^c	18.67 (± 1.20) ^d	12.67 (± 0.58) ^a	19 (± 0.58) ^c	13.33 (± 0.33) ^a	20.33 (± 0.58) ^b
PB - VII	11.67 (± 0.33) ^a	19 (± 0.58) ^d	18.67 (± 0.67) ^d	12.67 (± 1.33) ^a	19 (± 1.15) ^c	13.33 (± 0.33) ^a	20.33 (± 0.33) ^c

*Values are the mean of three replicates (± SE) at 100 µg/mL concentration. Means within the column followed by the same letter do not differ significantly as determined by Duncan's multiple range test ($P < 0.05$) among the treatments

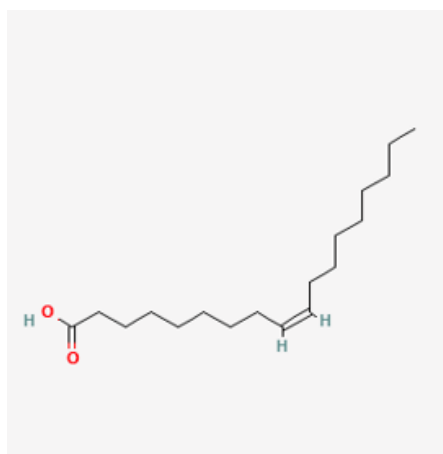
Alkaloidal fractions have shown significant antibacterial activity against the specified bacteria. In life sciences, alkaloids have been processed as large and diverse groups. Natural alkaloids, specifically from seed extracts have been extensively studied, showing that certain natural alkaloids like Diaboline can (i) disrupt cell membranes (ii) affect DNA function (iii) inhibit protein synthesis, as mentioned in the following reference. (NIH, Research Progress on Antibacterial Activities and Mechanisms of Natural Alkaloids: A Review, 2021).

1.2 *Moringa Oleifera*

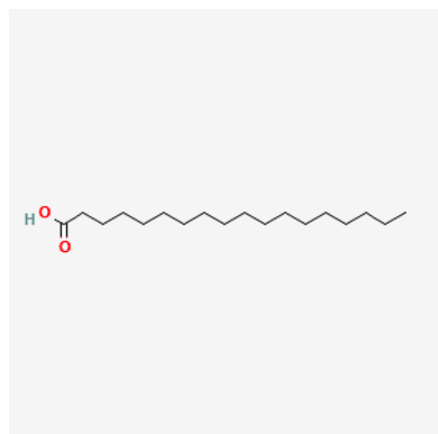
Moringa oleifera also known as the “Moringa leaves” is indigenous to Southeast Asia, specifically the Himalayas and southern India. It was later recalled as “The Medicinal Tree” because of its medicinal impacts, hosting benefits to modern Ayurveda as early as 2000 BC. (MoringaMix, Origin, 2016).

Moringa oleifera originates from the “Moringaceae” family and is commonly grown in wild forests. This plant was originally cultivated for its nutritional potential. In addition, the leaf extract has also shown antimicrobial activity against fungi and bacteria.

In the research paper “Chemical Analysis and Antimicrobial Activity of *Moringa oleifera* Lam. Leaves and Seeds” written by a group of scientists across the nation (PubMed, Analysis and Antimicrobial Activity of *Moringa* Leaves and Seeds, 2020), apolar and polar extracts of the *Moringa* Seed were taken and investigated it on. A high concentration of fatty acids such as Oleic Acid and Stearic Acid were identified in the extracts. Below are the respective chemical structures.



(PubChem, Oleic Acid, 2004)



(PubChem, Stearic Acid, 2004)

Oleic Acids and Stearic Acids can disrupt cell membranes, signifying their predominant antimicrobial properties. Both acids are strongly effective against several gram-positive bacteria, such as *S. Aureas* UAMS-1 Strain and *Bacillus megaterium*. (SD, Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*, 2000).

Hence, the antimicrobial properties of the *Moringa oleifera* have been experimented with various sorts of bacteria. However, this paper attempts to experiment with the properties of *Moringa oleifera* against various bacteria present in samples of contaminated water.

1.3 *Salvia hispanica*. L

Salvia Hispanica. L, also known as the “Chia Seed”, originated from Southern Mexico and Northern Guatemala. *Salvia Hispanica*. L has historic roots rising from the Mesopotamian cultures of the Aztecs and Mayas. It is a part of the order Lamiales, mint family Labiate, and the subfamily Nepetoidae. (NIH, *Salvia Hispanica* L.: Phytochemical Profile, Isolation Methods, and Application, 2019). Additionally, the seed has shown results in antimicrobial properties against gram-positive bacteria.

In a research paper called “Antibacterial peptide fractions from chia seeds (*Salvia hispanica* L.) and their stability to food processing conditions”, where 3 Chia Peptide Fractions were investigated (F < 1, F 1-3, and F 3-5 kDa) against *S. Aureas*, *L. monocytogenes*, and *B. subtilis* bacteria. The F<1 protein showed major inhibition against all bacteria. Furthermore, in the research paper “Antibacterial peptide fractions from chia seeds (*Salvia hispanica* L.) and their stability to food processing conditions”, F>1 showed longevity against gram-positive bacteria, and a more significant inhibition effect was reported against bacteria like *Listeria monocytogenes*.

Hence, the antimicrobial properties of *Salvia Hispanica* L. have been experimented with various sorts of bacteria. However, this paper attempts to experiment with the properties of *Salvia Hispanica* L, against various bacteria present in samples of contaminated water. This can include, as mentioned, the phenolic and humilic compounds present in water as well as colloidal materials.

1.4 *Foeniculum Vulgare* Mill

Foeniculum Vulgare Mill, also known as the fennel seed, originating from the southern Mediterranean region is an aromatic herb. It has been used in Eastern medicine since prehistoric times and as a flavor enhancer (Usage and Significance of Fennel (*Foeniculum vulgare* Mill.) Seeds in Eastern Medicine, 2010). *Foeniculum Vulgare* Mill is a part of the plant family known as Apiaceae or Umbelliferae, many plants of this family have been known to contain antimicrobial and medicinal properties, (Antimicrobial Activities of African Medicinal Spices and Vegetables, 2017).

Scientific Data indicates their effectiveness in various pharmacological properties such as antimicrobial properties (*Foeniculum vulgare* Mill: A Review of Its Botany, Phytochemistry, Pharmacology, Contemporary Application, and Toxicology, 2014).

A study titled, “Antimicrobial and antioxidant activities of fennel oil”, investigated the antimicrobial properties of the *Foeniculum Vulgare* Mill, it used a disc diffusion technique to evaluate the antimicrobial properties against four bacterial species (*S. aureus*, *P. aeruginosa*, *B. Subtilis*, and *E. coli*). The results show that the antibacterial activity of the essential oil is quite powerful. The observed effects aligned with Khaldun’s findings, which stated that fennel oil had a potent anti-bacterial effect on the fungus strain *Candida albicans*.

The three primary components of fennel essential oil were identified by GC analysis as trans-anethole (78.38-86.08%), methyl chavicol (2.32-2.54%), and fenchone (6.65-8.95%).

2. MATERIALS AND METHODS

2.1 Preparation and Seed Extraction:

Each seed sample was taken in 3 trials with a dirty water sample taken as the constant. All seed samples were grown and purchased from Local Ayurvedic Retailers. To prepare the respective extracts each seed was individually ground using a kitchen blender and 0.5 grams of each seed powder was stirred with 50 ml of methanol in an individual beaker and was rested overnight in a dark place.

An agar solution was prepared in excess by mixing 11.9 grams of agar powder in 425 ml of distilled water in a flask. The solution was then boiled to ensure the powder dissolved completely using a hotplate. 13 glass Petri dishes were selected and rinsed with tap water. Newspaper was used to wrap the Petri Dishes and both the Agar and Petri Dishes were kept in an autoclave for 90 minutes.

After 12 hours, the extracts were removed from the dark area and 10 ml of seed extract was taken from each sample and mixed with 90 ml of dirty water that was acquired from the local Gandipet Lake. The samples were stirred and kept to rest for 30 minutes.

2.2 Spreading of Bacteria, Incubation, and Colony Counting.

25 ml of agar was poured onto each petri dish and using a micropipette, 100 microlitres of each seed extract was transferred onto each of the 13 petri dishes except for the dirty water sample which was used as a control dish. A bacterial cell spreader was used to spread each sample of seed extract onto the respective petri dish for 3 trials as each extract was tested with 3 Petri dishes for 3 trials.

The Petri dishes were then placed in a "Bacteriological Incubator Memmert Type" Incubator for 24 hours. After 24 hours, the petri dishes were removed from the incubator. The number of bacterial colonies on each petri dish, for each trial of the seed extracts, was counted using a Manual colony counter LMCC-A11. Countable colonies were identified as shown in Figure 3.1

3. RESULT

Type of Seed Extract	Colony Count/100 μ L			Mean # of Colonies
	Trial 1	Trial 2	Trial 3	
Strychnos Potatorum	259	216	198	224.33
Moringa Oleifera	554	517	584	551.67
Salvia Hispanica	447	431	473	450.33
Foeniculum Vulgare Mill	310	346	298	318
Dirty Water	1056			1056

Fig 3.1

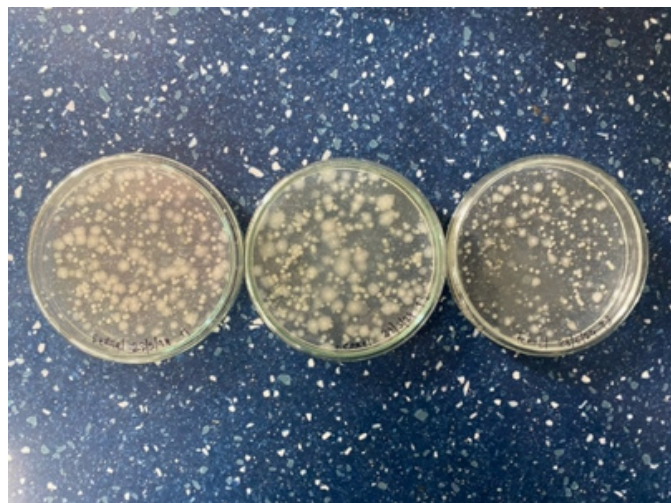


Fig 3.2 *Foeniculum Vulgare Mill*

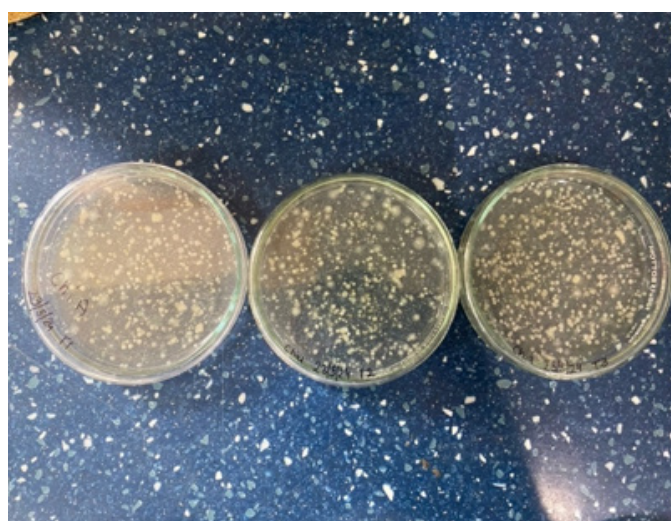


Figure 3.3 *Salvia Hispanica*

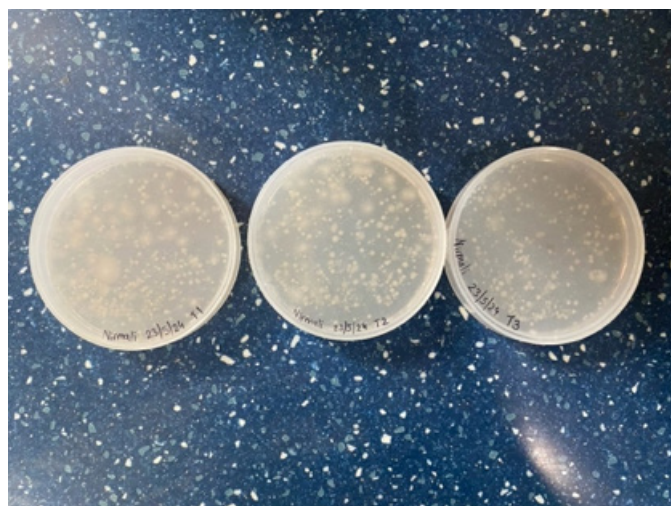


Figure 3.4 *Strychnos Potatorum*

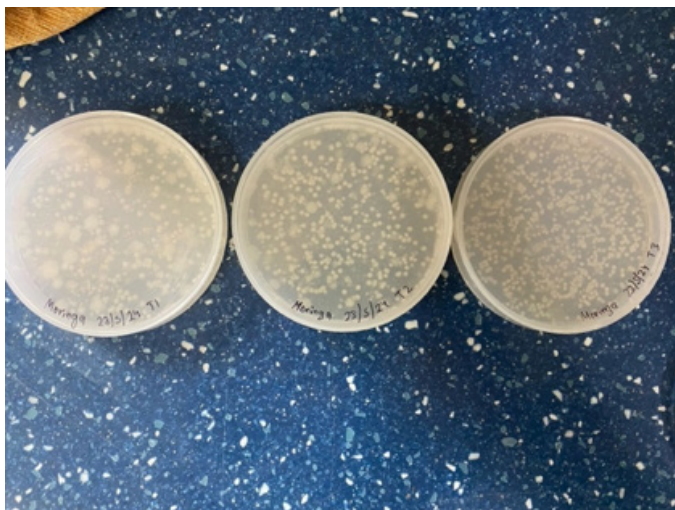


Figure 3.5 *Moringa Oleifera*

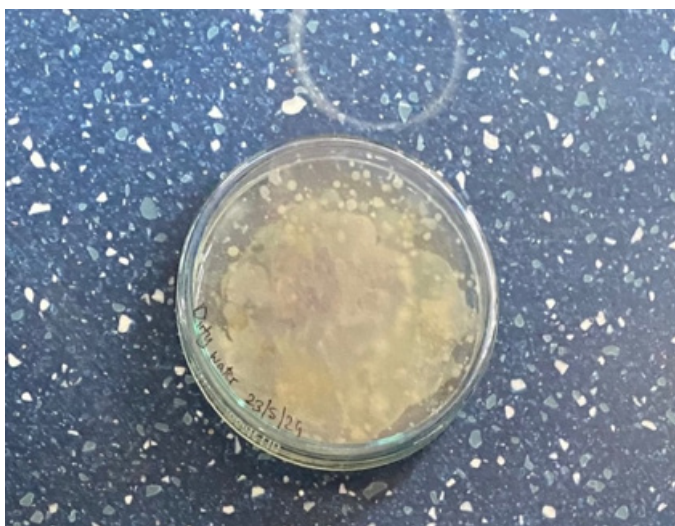


Figure 3.6 Dirty Water

4. DISCUSSION AND DATA ANALYSIS:

4.1 Qualitative Analysis:

Figure 3.4 contains images of the *S. Potatorium* samples which show that the bacterial colonies are sparser and larger. They are spread widely across the dish and have larger diameters compared to the *S. Hispanica* and *M. Oleifera* seed samples. T1 is shown to have the densest and most populated growth while T3 contains microbial colonies that are sparsely spread apart. All 3 samples of *S. Potatorium* have shown to have similar results in terms of visible microbial colonies.

With the *M. Oleifera* samples, we identified an increased value of bacterial colonies with a smaller diameter, they were more spread out and did not accumulate at a certain location. *M. Oleifera* has the largest amount of visible colonies out of the 4 samples and is shown to be the most densely populated. In Figure 3.5, T3 is shown to be much more densely populated than T1 and T2. Figure 3.5 also shows that each bacterial colony has a smaller diameter of around 1-2mm while T1 and T2 have colonies of around 4-5mm.

With the *S. Hispanica* seed samples, we were able to identify

a large number of bacterial colonies with tinier diameters compared to the other 3 seed extracts, they weren't grouped up and were comparatively more spread out and evenly distributed inside the Petri dish. Figure 3.3 shows that T3 is the most densely populated with little distance between each bacterial colony, T3 is shown to have a few bacterial colonies that are significantly larger than the other colonies in the petri dish. T1 and T2 have similar results and they are shown to have a distance between each of the colonies.

Figure 3.2 demonstrates images of the *F. Vulgare Mill* samples, these samples are shown to have larger colonies compared to the other 3 seed samples, many of the bacterial colonies reach up to 6mm and are sparsely distributed throughout the petri dish. T2 shows the accumulation of many of these larger colonies and is shown to have large amounts of space between each colony. T1 has similar results to those in T2 however, there are numerous amount of smaller colonies, the bigger colonies do not have the same diameters as those in T2 and T3, but there are a larger amount of these colonies.

4.2 Quantitative Analysis:

Based on the results above, from Fig 3.1-3.5, *Strychnos Potatorium* showed the greatest bacterial inhibition, followed by *Foeniculum Vulgare Mill*, *Slavia Hispanica*, and *Moringa Oleifera*. Compared to dirty water, the seeds inhibited what was nearly **500-700 colonies of bacteria** that could have been present in the samples.

S. Potatorium samples inhibited 78.76% of bacteria present in Dirty Water. *F. Vulgare Mill* samples inhibited 69.7% of bacteria present in Dirty Water. SH samples inhibited 57.46% and *M. Oleifera* samples inhibited 47.76% of bacteria in dirty water. This quantitative analysis indicates that *Strychnos Potatorium*** and ***Foeniculum Vulgare Mill* are the most promising seed extracts for reducing bacterial contamination in water.

4.3 Discussion

The study of various phytochemicals present in different seed samples can help us learn about the new functions of life and what they can aid towards. When looking at different seeds such as (i) *Strychnos Potatorium*, (ii) *Moringa Oleifera*, (iii) *Salvia hispanica*, and (IV) *Foeniculum vulgare Mill* we were able to test and identify the ability of these seeds to inhibit bacteria in dirty water. This shows that various seeds present in nature can purify wastewater and reduce the threat of these bacteria when consuming water.

In this investigation, we conducted antimicrobial tests on 4 different types of seeds to find out if they are potent in inhibiting wastewater bacteria such as *Proteus vulgaris*, *Staphylococcus aureus*, and *Salmonella typhimurium*. By taking wastewater samples from Gandipet Lake. A colony counting test was conducted and the results from Fig 3.1-3.5 indicate the strong bacterial inhibiting potent of these seeds.

Further investigations can aim towards isolating the specific phytochemicals responsible for coagulation/antimicrobial properties. The isolation of these phytochemicals can contribute

towards an overall product that introduces portable or feasible waste water purification. Such investigations can also contribute towards the United Nations Sustainable Development Goal No. 6 which states “Clean Water and Sanitation”.

The characterization of various phytochemicals in seeds can further advance our data in PBD banks or other such banks and improve our understanding of the different functions of phytochemicals and their role in life sciences and biochemistry.

5. CONCLUSIONS

The antimicrobial and antibacterial properties of 4 different seed extracts (i) *Strychnos Potatorium*, (ii) *Moringa Oleifera*, (iii) *Salvia hispanica*, and (IV) *Foeniculum vulgare* Mill have been tested against dirty water samples with 1000+ visible colonies. Out of the 4 seed extracts, *Strychnos Potatorium* has been shown to have the strongest antimicrobial properties in terms of wastewater filtration, with an average of 224.33 visible colonies in 100 µL of dirty water. The alkaloid Fractions present in the seed such as *Diaboline* and *Dolichantoside* were able to increase the floc size helping aid in the coagulation process. The increased amounts of humilic acid and phenolic compounds also improved the floc size to help increase and aid the coagulation and the formation of colonies in *S. Potatorium*.

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